Bermann (I.)

SOME POINTS ON STAINING *IN TOTO* AND DRY SECTION CUTTING

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SOME POINTS ON STAINING *IN TOTO* AND DRY SECTION CUTTING.

By I. BERMANN, M.D.,

BALTIMORE, MD.

THERE are so many different methods given in the hand-books for microscopical technique, that it may almost appear superfluous to add a new (at least in some respects new) one, to those already in existence. Still, these do not always prove satisfactory, an account of their requiring too much time, and as my method of staining *in toto* and dry cutting seems to be only known to the few who have seen me make use of it, and who have expressed a desire that I should publish a description of it, I do so, more particularly for those medical men who wish to continue those studies—particularly of pathological microscopy—indispensable to a truly scientific education. It is especially adapted to them, as it enables them to work whenever they find leisure, and to leave off at any stage of the proceedings, without fear of spoiling what is unfinished.

For hardening the tissues—with scarcely an exception—Müller's fluid (containing two and a half parts bichromate of potash, one, to one and a half parts sulphate of soda, and one hundred parts distilled water) is to be preferred, because it hardens the tissues without shrinkage, and almost preserves their natural appearance.

It may not be amiss here to mention that it can be satis-

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factorily employed only when the following rules are strictly observed: First, the greater the quantity of fluid —within certain proportions to the size of the specimen to be hardened—the more confidently one may count upon its success.

Secondly, the fresher the specimen the more accurate and reliable will be the definition of the cellular construction of the sections.

Specimens not prepared after this fashion will, under no circumstances, repay the labor and trouble expended upon them.

Besides slightly staining the tissue, Müller's fluid has the advantage of preserving the blood corpuscles in the blood-vessels better than any other hardening agent, but to attain this end, the fluid, which is perfectly transparent, should not be allowed to lose its transparency, and must be very frequently changed—not less than twice on the first day, and then (according to the quantity of the fluid) at least every other day. A small piece—say half a cubic inch—requires about a week to attain the right consistency, when, after first washing it in water, it is transferred into alcohol of about 75 per cent. It is not absolutely necessary to harden the specimen in Müller's fluid first; those put only in alcohol in the beginning will stain just as well. I prefer to use Müller's fluid, because most tissues shrink in alcohol unequally, and thus are apt to spoil the right proportions of the specimen.

Specimens hardened in a two-per-cent. solution of bichromate of ammonia, when proper care is taken that the hardening fluid is changed frequently and the alcohol is perfectly colorless, when put into the staining fluid, will also give very nice results.

To insure perfect success in staining it *in toto* (one of the principal advantages of my method), the alcohol

must be changed as often as it becomes yellow, and the specimen is not ready for staining until the alcohol remains perfectly colorless. The specimen is then removed to the staining fluid, the formula for which I published first in 1878*, which is as follows: Dissolve 5 grammes of best carmine in about 8 grammes of caustic ammonia, then add distilled water until its contents equal 100 cubic centimetres. After equal parts of absolute alcohol and glycerine are added to it, it is put on the hot water bath and kept there until the prevailing odor of the ammonia has almost disappeared, which is usually accomplished in about two hours, and when properly made no sediment will be deposited. The fluid is now ready for use, and has this great advantage over other carmine solutions, that it can be used over and over again, and at the same time acts as a preserving fluid, so that the specimen can be left in it for an *unlimited time*. It never stains too deep a shade, and makes the nuclei come out very sharp. For the examination of glands, where it brings out the lunulæ most perfectly, and also of nervous matter, it can not be surpassed. The disagreeable quality of carmine, to stain well one day and diffuse the next, is thereby entirely obviated.

Another staining fluid with which I have obtained beautiful results, either in combination with the first for double staining, or by itself alone, is that prepared with hæmatoxyline. It is especially to be recommended for pathological specimens, because the hæmatoxyline has a peculiar affinity to the products of inflammation and cells of pathological origin.

I prepare this fluid by mixing Bœhmer's hæmatoxyline*

* Ueber die Zusammensetzung der glandula submaxillaris aus verschiedenen Drüsenformen und deren functionelle structur veränderungen. Würzburg, 1878. F. Staudinger.

* Bœhmer's solution for staining sections is prepared as follows: 1 part crystallized hæmatoxyline is dissolved in 30 parts (weight) of absolute alcohol,

with equal volume parts of absolute alcohol and glycerine. Thus mixed it forms the solution for staining *in toto*. It does not stain so quickly as the carmine solution, and pieces of the afore-mentioned size must remain in it from three to four weeks, when they are taken out and again put into alcohol to harden.

To return to the treatment of specimens stained in the carmine solution alone. They are taken out and, to remove the superfluous carmine, are washed in distilled water for a few hours, then put into weak alcohol to be changed for stronger till no more of the pink coloring matter is drawn from the specimens; then to extract all water from the tissue it is transferred into absolute alcohol, which must be changed several times.

When this end has been accomplished, it is put into spirits of turpentine, also to be renewed twice at least, for large pieces. It is then placed in a saturated solution of paraffin in spirits of turpentine, where it is again left for several days—the time varying with the size of the specimen. Next it is removed and laid on filtering paper, that the turpentine may evaporate; the paraffin remains and fills the crevices, blood- and lymph-vessels, etc. When sufficiently dry, it is embedded in a mixture of paraffin and mutton suet, the proportions of which vary with the seasons. In summer pure paraffin will be found necessary.

The paraffin should always be heated on a water bath, and it will be found very convenient to throw the piece to be embedded into the hot fluid mixture, so that it gets thoroughly soaked, and when cooled off, forms an inseparable mass of equal consistence with the paraffin. I have

1 part chemically pure alum in 30 parts (weight) of distilled water; then so much of the alcoholic hæmatoxyline solution is poured into the alum solution (stirring it all the while with a glass rod) till it changes its color to a dark but transparent violet. It is then left for twenty-four hours in the dark, where a slight sediment will form, which must be filtered off to make the hæmatoxyline fluid ready for use.

never found any tissue, not even embryonical, to spoil by exposing them to a moderate heat, while in the fluid paraffin.

It is then ready to be cut *dry*, and can be preserved in this state any number of years.

For cutting it I employ a sledge microtome, made by Haertel, in Breslau, after designs by Dr. Long, which enables me to make very long sections of equal thickness—from $\frac{1}{100}$ of a millimetre upward. When properly constructed, it is the most perfect instrument of its kind I know, and the cost of it, with two knives and with case is, in Germany, at the makers, seventy-five marks, equal to about nineteen dollars. The section thus cut is transferred to a slide where it frequently has to be unrolled—the only drawback to the method, as it requires some dexterity of manipulation. To dissolve the paraffin remaining around and in the section, a few drops of solution of creosote and turpentine (in the proportion of one to four) are added, and when this has been removed from the glass by wiping, or by drawing it up with filtering paper, it is enclosed in either Damar varnish or Canada balsam.

It happens pretty frequently that the specimen, when in the paraffin, is found not to adhere to it; in that case I throw the whole piece back into the hot paraffin and suet mixture and embed it then anew. By this manipulation I get rid of the superfluous turpentine, which was the cause of my former poor success.

By following this method to the letter, it will be certain to reward any co-laborers in microscopical research, and they will obtain especially fine specimens by staining first in the carmine, and then in the hæmatoxyline solutions. These double-stained specimens enable us to differentiate the individual cells, which take the carmine solution up with equal intensity. As a matter of course, the specimen

stained in carmine has first to be washed out and hardened in alcohol, before subjected to the hæmatoxyline solution. For instance, the neuroglia cells of the spine and brain, cancer cells and other pathological products are brought out with greater clearness.

Another method of staining *in toto*, which was, as far as I remember, first published by Kleinenberg, gives frequently, especially for tumors containing ossificated tissue, very nice results. The specimen is first decalcinated by a concentrated solution of picric acid, which takes several weeks, and then, without being washed out, dropped into an ammoniacal solution of carmine. There it is left for twenty-four hours and then hardened in alcohol. By then subjecting it to the same process as described, it can also be cut dry. For delicate tissues it will be found not to answer very well, as it causes frequently considerable shrinkage.

I obtained the best results with it on brain tissue, nerves, etc., but never equal to those stained with the carmine solution.

It may hardly be necessary to state, in conclusion, that objects thus stained *in toto*, can be preserved in alcohol for an unlimited time, and can also be embedded for cutting in any other manner, according to the fancy of the worker.

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